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TRANSEPIDERMAL ACCUMULATION OF NATURALLY OCCURRING AMINO ACIDS IN THE SAND DOLLAR, *DENDRASTER EXCENTRICUS* [1]

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Echinoids and asteroids exhibit a full range of feeding habits including carnivores, herbivores, detritus feeders and filter feeders. However, distribution of nutrients derived from digestion, whatever the feeding habit, appears to be slow and incomplete. Ferguson (1970) injected a mixture of ^{14}C-labeled amino acids into the stomach and perivisceral coelom of the starfish, *Echinaster,* and followed the subsequent distribution of labelled material using autoradiography. Translocation of nutrients throughout visceral and subepidermal regions was observed, but no labelling of epidermal tissue was evident at the end of 75 days. Conversely (Ferguson, 1967), ^{14}C-labeled amino acids supplied in the ambient medium were incorporated into epidermal tissues of starfishes, but there was little or no export of label from epidermis to subepidermal or visceral tissues. A comparable barrier to distribution of nutrients between epidermal and visceral tissues is discussed in the work Péquignat (1969, 1970), Péquignat and Pujol (1968), and Pearse and Pearse (1973) employing various echinoids and asteroids. The barrier is not necessarily complete. Slow translocation across the barrier is reported by some investigators. These observations agree well with the morphology of these echinoderm groups; the "circulatory" systems, though complex, do not appear to provide a well-organized morphological substrate for distribution of material to the epidermis.

Stephens and Schinske (1961) showed net influx of glycine from a rather concentrated solution into two species of starfishes. Since that time, a number of investigators have studied uptake of amino acids in echinoderms (*e.g.,* Stephens and Virkar, 1966; Fontaine and Chia, 1968; Clark, 1969; Dixit, 1973; Ahearn and Townsley, 1975). Most of this work used ^{14}C-labeled substrates and described kinetics of influx by radiochemical techniques and/or distribution of labeled material by autoradiography. However, Ferguson (1971) showed by direct chemical determination that there was a net influx of amino acids from a medium concentration of 37.5 μM into ten different species of starfishes from the Puget Sound area. This work did not permit estimation of rates of net influx but established the capacity of several of the forms employed to reduce ambient amino acid concentrations to extremely low levels at the end of a six-hour incubation period.

There is, thus, considerable evidence for uptake and utilization of amino acids by epidermal tissues in echinoids and asteroids. There is also considerable evidence

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that distribution of nutrients from the digestive system to superficial tissues is very slow. This has led Ferguson (1970) to suggest that epidermal tissues may derive much of their sustenance by direct influx of nutrients from the environment independent of the nutrition of visceral and subepidermal tissues. Péquignat (1970) frames a similar hypothesis, adding the possibility of cutaneous digestion and assimilation of larger organic substrates to account for support of epidermal structures.

The sand dollar, *Dendraster excentricus,* was selected as an experimental organism for two reasons. First, there are large populations of this organism readily available for study and the feeding behavior has been well described (Timko, 1976). Secondly, we wanted to work with an organism which lives in or on a soft substrate, since the occurrence and distribution of amino acids in such habitats has already been analyzed (Stephens, 1975) in relation to the nutrition of annelid infauna.

In the present work, data are presented on the following: kinetics of influx of ^{14}C-labeled amino acids from solution, kinetics of net influx of amino acids, levels of amino acid present in the microenvironment, availability of naturally occurring amino acids as assessed by net influx, estimates of energy metabolism of whole animals, and estimates of energy metabolism in isolated portions of the test. Collectively, this information allows an estimate of possible contributions of transepidermal transport of amino acids to the support of epidermal tissues.

Materials and Methods

Animals and sediment samples were obtained from two locations. Most of the material studied was obtained from a shallow water population in a lagoon at Point Mugu Naval Base near Port Hueneme, California. Some samples were taken from a population off Newport Beach at a depth of approximately 5 meters. Animals were maintained in aerated sea water at a temperature of 15° C. Sediment cores were taken using plexiglass coring tubes (25 mm internal diameter) and were analyzed promptly. Interstitial water from sediment was expressed through a Millipore filter (0.45 μm) under nitrogen at 10–20 psi.

Influx rates of amino acids were determined by measuring the disappearance of radioactivity from a solution containing the ^{14}C-labeled compound. Solutions were prepared in artificial sea water (Cavanaugh, 1956) prepared from reagent grade salts. Radioactivity was initially 20 μCi/liter ($2–5 \times 10^{-7}$ moles/liter depending on specific activity) with ^{12}C-amino acid added to obtain the desired concentration. Radioactivity was measured using a scintillation counter; samples of 0.5 ml were added to a toluene-based cocktail containing a detergent to solubilize the sample. Samples were acidified to drive off CO_2. Volumes to which animals were exposed ranged from 50 to 200 ml; air was bubbled slowly through the vessel to provide aeration and circulation.

Net flux of amino acids was followed using fluorescamine (North, 1975) to determine primary amines in solutions to which the animals were exposed. After sample preparation, fluorescence was measured using a Perkin-Elmer spectrophotofluorometer with an excitation wavelength of 390 nm and an emission wavelength of 480 nm. This procedure was also used for estimating levels of naturally occurring primary amines in interstitial water of sediment samples. Some of the

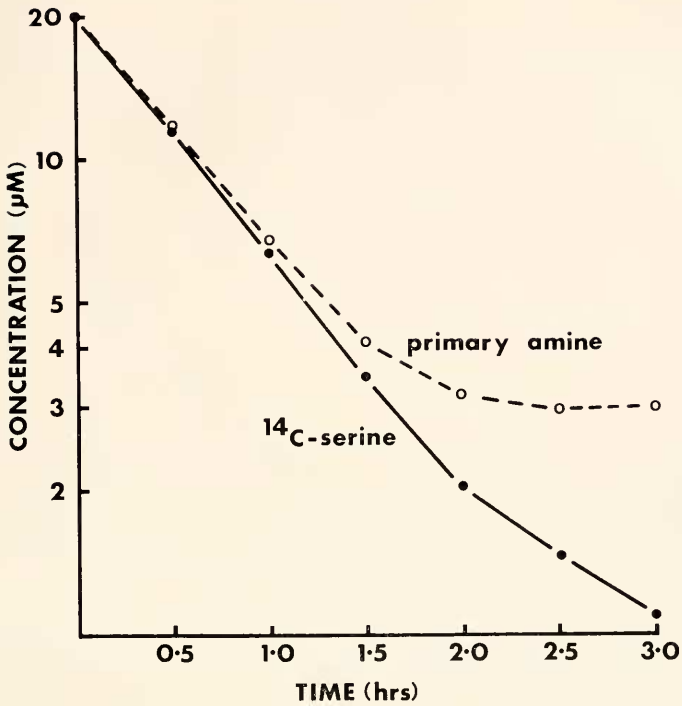

FIGURE 1. Removal of serine from 100 ml of a 20 μM solution by a specimen of *Dendraster*. The solid line shows decrease in radioactivity with time; the broken line shows decrease in primary amines estimated by the fluorescamine procedure.

latter measurements were repeated using an independent procedure based on o-phthalaldehyde (OPA). The procedure is based on that of Mendez and Gavilanes (1976) but employs a lower concentration of OPA (0.03 mg/ml rather than 0.3 mg/ml). It proved to be necessary to read samples and standards at a constant time after preparation.

Amino acids present in interstitial water before and after exposure to the animals were identified by thin layer chromatography. Seawater samples (5–10 ml) were desalted on Dowex–50, eluted off the column with 3 N NH_4OH and chromatographed as described by Clark (1968) with the following modifications. Spots (1–5 μl) were applied to the chromatogram using a glass capillary drawn to a tip diameter of approximately 50 μm which minimized spot diameter. Chromatograms, 10 cm × 10 cm, rather than 20 cm × 20 cm as supplied (Polygram CEL 300), were developed for approximately one hour in each dimension without scoring following the first solvent system.

Spots were located using OPA as a location reagent as follows. An OPA stock solution is prepared several hours prior to use by dissolving 30 mg OPA in 200 ml glass distilled water and adding a drop of 1 N NaOH. This stock is stable for one to two days. Just prior to use, the spray mixture is prepared consisting of 20 ml OPA stock, 20 ml absolute ethanol, 0.2 ml triethylamine and 0.01 ml 2-

Table I

Rates of disappearance of amino acids expressed as nmoles/(hr·cm²). Data for aboral surface and for both surfaces are combined. Average diameter 4.1–7.6 cm.

	Mean rate	Standard deviation	N
ala	42.0	2.7	2
asp	16.8	2.3	3
gly	45.8	5.0	6
glu	6.1	1.0	5
lys	25.4	3.8	5
ser	49.5	5.5	8
val	63.0	4.6	3
naturally occurring primary amines	24.9	7.6	5

mercaptoethanol. The spray mixture is stable for several hours. It is applied using a mist sprayer; approximately 5 ml suffices for a 10 cm × 10 cm plate. One to ten minutes after spraying, chromatograms are examined under a long wavelength UV-lamp. Most spots intensify on drying, but lysine fades. Detection levels for most amino acids range from 20 to 50 picomoles. However, 200 to 300 picomoles are required for location of some hydrophobic amino acids (*e.g.,* val, leu, ile). Chromatograms were photographed through a yellow filter (455 nm) using Kodak Tri-X film and diafine development (ASA 1600) at f5.6 and 0.5 second exposure. Schiltz, Schnackerz and Gray (1977) have recently described a comparable procedure. When chromatograms are prepared as described, ninhydrin can also be used as a location reagent with detection levels in the range of a few hundred picomoles.

Oxygen consumption was measured using a YSI oxygen electrode. Measurements of oxygen consumption as well as studies of influx and net flux of amino acids were carried out at a temperature of 20° C.

Results

Figure 1 presents the results of a typical set of observations. A sand dollar, 7.2 cm average diameter, was placed in 100 ml of artificial sea water to which serine had been added at a concentration of 20 μmoles/liter. The solution also contained 2 μCi of ^{14}C-serine (UL). As indicated in the figure, radioactivity decreased rapidly with time as did the total primary amine in the solution estimated by the fluorescamine reaction. Disappearance followed first order exponential kinetics for the first hour, and the two curves are virtually identical. The rate of entry (disappearance of primary amine) at the initial concentration of 20 μM was 2.15 μmoles/hr. It proved to be best to relate rates to the surface area of the animals and express them as nmoles/(hr·cm²). This gave consistent results over the considerable size range of animals examined. For the case presented in Figure 1, uptake of serine from a 20 μM solution proceeded at a rate of 53 nmoles/(hr·cm²). If influx of ^{14}C is expressed in the same units, the rate from a 20 μM solution is 56.7 nmoles/(hr·cm²).

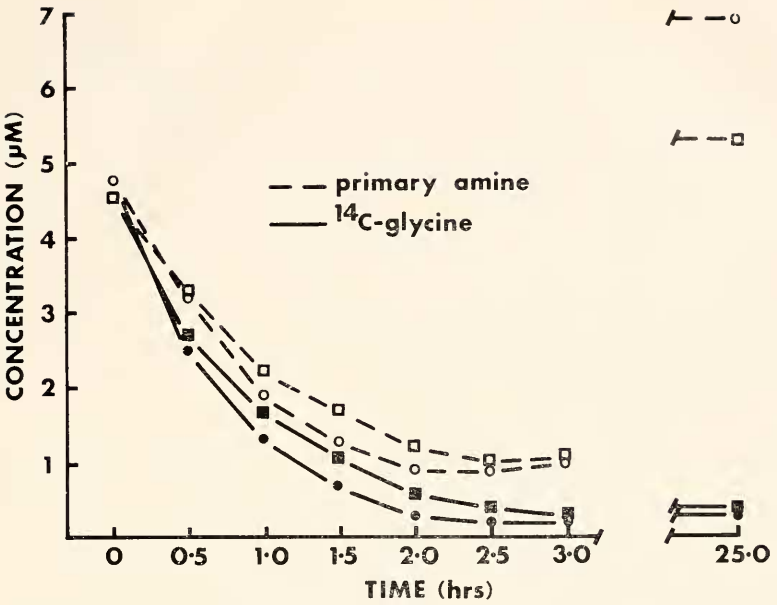

FIGURE 2. Removal of glycine from 100 ml of a 5 μM solution by two specimens of *Dendraster*. Data points for the two individuals are solid and open circles and solid and open squares respectively.

Results of experiments of this kind were quite repeatable. When animals were supported on glass rods to facilitate circulation of solution across the oral surface, observed rates of disappearance of an added amino acid were approximately doubled. Thus both surfaces of the animals seem to participate equally in removal of amino acid from dilute solution. Table I presents rates of disappearance of various amino acids. All rates are expressed as nmoles/(hr·cm²) at an ambient concentration of 20 μM. It should be noted that the determinations of ^{14}C and of primary amines diverge at low concentrations (Fig. 1). Figure 2 shows data for two different animals offered ^{14}C-glycine at 5 μmoles/liter. Fluorescent material declined over the course of two hours to approximately 1 μmole/liter (glycine-equivalent concentration) and then slowly increased to levels of 5–7 μM over the course of the ensuing 23 hours. If animals were placed in a small volume of artificial sea water with no added amino acid, primary amines slowly increased and stabilized at similar final concentrations. These same levels were found in the aerated water in which groups of animals were kept over a period of days.

Figure 2 also shows that small amounts of radioactivity (6–8%) remained in the solution 25 hours after ^{14}C-glycine was supplied. In the case of other amino acids, for example serine, this effect was quite pronounced with as much as 15–20% of initial radioactivity persisting in solution at the end of 24 hours. The radioactivity does not appear to be in the form of serine; about 60% of the activity passes through a Dowex-50 column in the acid form, and TLC shows several spots that are unidentified but do not react as primary amines.

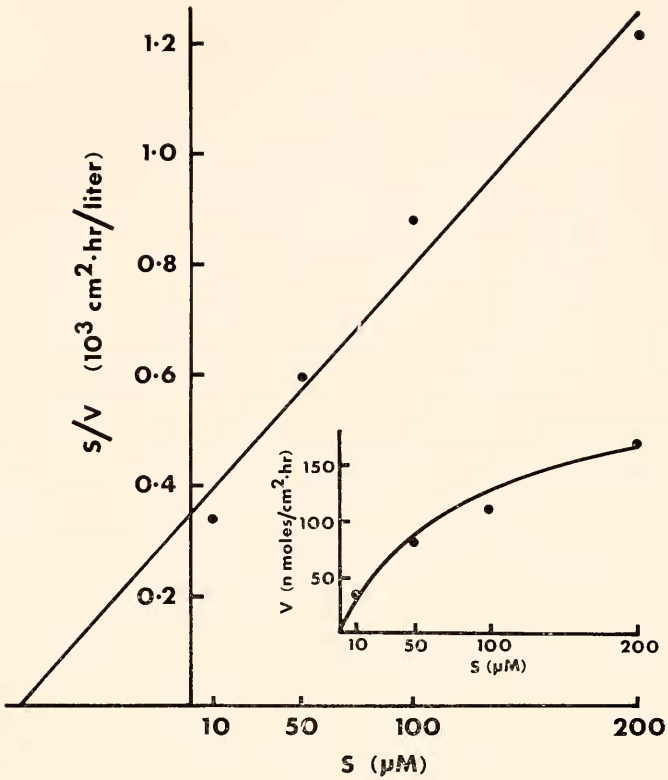

FIGURE 3. Removal of glycine from solution by *Dendraster* as a function of ambient concentrations. The insert graph presents rates determined at the concentrations indicated; the curve is a hyperbola fitted to the kinetic constants. The larger graph is a Woolf plot of the data. K_t is 74 μM V_{max} is 215 nmoles/(hr·cm²).

Figure 3 presents data relating influx (measured by disappearance of ^{14}C) to glycine concentration. The insert is a plot of influx as a function of ambient concentration. Kinetic constants were evaluated from the Woolf plot presented in Figure 3. The K_t was 74 μM and the V_{max} was 215 nmoles/(hr·cm²).

Dendraster does not take up glycylglycine from dilute solution. Animals were incubated for 24 hours in 20 μM glycylglycine with a trace amount of ^{14}C-glycine. Radioactivity in the medium decreased rapidly, as expected. Fluorescamine positive material (expressed as equivalent glycylglycine concentration) increased slightly during early incubation, presumably reflecting efflux of unknown primary amines. After 24 hours, levels had decreased to about 75% of the original concentration. This may reflect microbial activity or very slow uptake. In any case, uptake of glycylglycine either does not occur at all or is so slow as to be insignificant compared to uptake of neutral amino acids.

Naturally occurring primary amines in interstitial water were determined using the fluorescamine technique and, in some cases, OPA. Determinations using the

two procedures were in good agreement. Sediment cores were divided into 3 cm zones from the surface downward; water was expressed through a Millipore filter under N_2, and concentrations of primary amines expressed as glycine-equivalent concentration. We do not believe that the cores taken at 5 meters depth were undisturbed. They showed interstitial concentrations of 17 and 23 μmoles amines/liter, respectively, in the top 3 cm. Core samples could be taken in the immediate vicinity of the shallow water population with minimum disturbance of sediment organization. The samples showed great variability in primary amine content. Fifteen samples gave an averaged value of 115 μM in the interstitial water of the top 3 cm of the cores with a standard deviation of 60 μM. The range was 17–244 μM. Stephens (1975) also reports considerable variability in primary amine concentration in sediment cores. In general, primary amine concentration decreased with depth, also in agreement with Stephens (1975) and Crowe, Dickson, Otto, Colón and Farley (1977), though there were two cores which showed an increase at the 3–6 cm and 6–9 cm zones.

Observations were carried out on rates of influx using samples of naturally occurring primary amines from both collection sites. Although the samples were expressed from sediment which was collected as carefully as possible, it is likely that they were somewhat diluted during collection. Also, a period of several hours elapsed before it was possible to obtain interstitial water from sediment collected at the shallow water site. The initial concentrations for the two sets of observations were 14 μM (for the deeper population) and 33 μM. The results are presented in Table I, recalculated to present rates from an ambient concentration of 20 μM to facilitate comparison with rates for known amino acids. The correction was made assuming a linear relation between ambient concentration and influx over the relevant range (14–33 μM).

Figure 4 presents photographs of TLC, including a standard and samples of sea water before and after exposure to a sand dollar for 24 hours. The standard contained 250 picomoles of each amino acid. The sea water to which the animal was exposed was interstitial water which initially contained 33 μM primary amine as estimated by the fluorescamine procedure. Final concentration was 7 μM. Desalted samples representing 125 μl of the interstitial water before and after exposure were spotted and chromatographed. Figure 4 illustrates the marked decrease in neutral amino acids at the end of the exposure period. A larger amount of the post-exposure sample was spotted and chromatographed; spots were more intense, but the pattern was the same as that illustrated. Neither the initial nor the final sample chromatograph in Figure 4 should be interpreted as a complete inventory of primary amines in the interstitial water. Only 70–85% of primary amine as estimated by the fluorescamine procedure is retained on passage through a Dowex-50 column in the acid phase and subsequently eluted with NH_4OH, whereas the retention of a standard mixture of amino acids in sea water is virtually complete. Thus, some of the naturally occurring primary amine is not behaving as do most amino acids, is not present in our desalted sample, and hence is not represented on the TLC. As an example, taurine reacts with fluorescamine but is not retained on a Dowex column. However, primary amines which pass through the column were not identified.

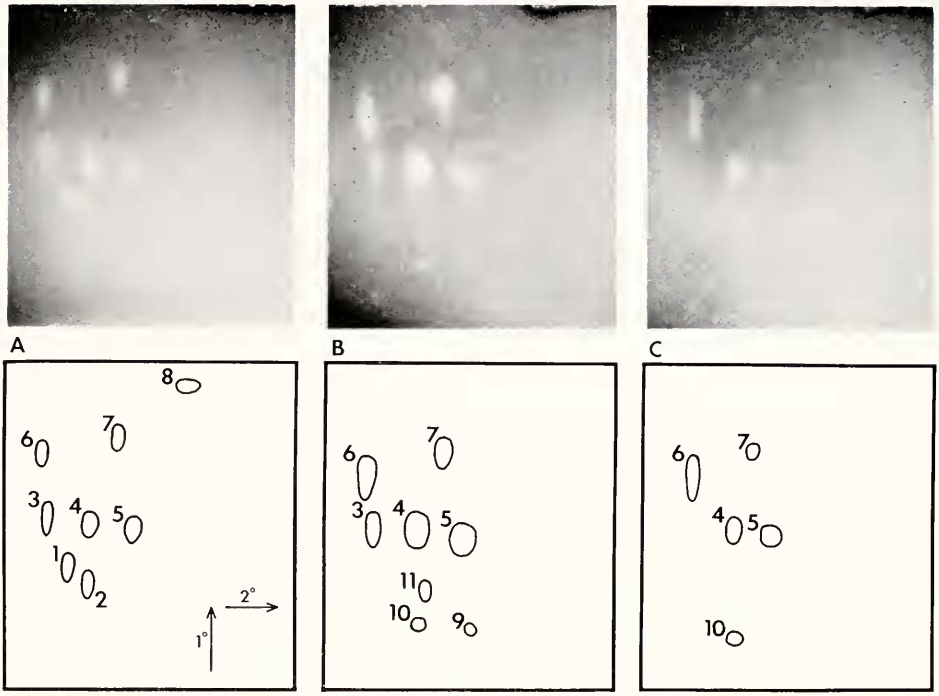

FIGURE 4. Thin layer chromatograms developed with OPA (see text for procedure). Amino acids are coded as (1) arg, (2) lys, (3) asp, (4) gly, (5) ser, (6) glu, (7) ala, (8) val, (9) his, (10) orn, (11) gln. **A** is a standard containing 250 picomoles of each of the first 8 amino acids, made in artificial sea water, desalted and run. **B** is a sample of interstitial water; total primary amine content is approximately 4.1 nanomoles. **C** is a sample of interstitial water after 24 hours exposure to a sand dollar; total primary amine content is approximately 875 picomoles. His, orn and gln are identified by Rf values from other standards.

Values for oxygen consumption were not found for sand dollars in the literature. The measurements presented in this study are intended to offer an approximate figure for oxygen consumption for comparison with measured rates of amino acid uptake. Two small animals (1.8 and 1.9 cm average diameters) consumed 4.6 and 4.7 μl $O_2/(hr \cdot cm^2)$; two large animals (6.15 and 5.75 cm average diameter) consumed 5.0 and 2.8 μl $O_2/(hr \cdot cm^2)$. In contrast to the relative constancy of oxygen consumption expressed per unit surface, oxygen consumption per unit weight decreased rapidly with size as would be anticipated. For the small animals, rates were 46.0 and 43.4 μl $O_2/(g \cdot hr)$; for the larger animals, they were 13.2 and 15.1 μl $O_2/(g \cdot hr)$. Despite the small sample size, it seems reasonable to accept the average figure of 4.3 μl $O_2/(hr \cdot cm^2)$ as an estimate of typical oxygen consumption at 20° C. Isolated portions of the aboral test survived well in aerated sea water for two to three days at 20° C as judged by general appearance and activity of pedicellariae. Oxygen consumption of two such portions of the test was 6.3 and 3.7 μl $O_2/(hr \cdot cm^2)$, respectively. The subdermal portion of the test was cleaned of adherent tissues, but the epithelium contributed to oxygen consumption; however,

it appears that the epidermis is responsible for a large fraction of the total oxygen consumption of the animals.

Discussion

Simultaneous measurement of influx and net influx of seven amino acids indicate that neutral amino acids (ala, gly, ser, val) are removed rapidly from dilute solution in ambient sea water by *Dendraster*. The amino acids asp and lys enter more slowly; glu is removed from solution very slowly, if at all (Table 1). Entry rates as estimated by disappearance of ^{14}C-labeled substrate and by chemical determination of total primary amine remaining in solution are comparable at ambient concentrations of 5 μM or more (Fig. 1). Thus, estimates of influx (^{14}C) reflect net influx (primary amine) at concentrations which are normally present in the habitat of the organism.

When *Dendraster* is placed in a fixed volume of sea water, an efflux of primary amines of unknown composition occurs until an apparent steady state is reached at an ambient concentration of 5–7 μM. Efflux appears to be slow compared with uptake of neutral amino acids. Thus, the pattern of primary amine concentration in the medium with time may show a decrease with a subsequent increase (Fig. 2).

Dendraster is capable of net accumulation from solution of some of the naturally occurring primary amines found in the interstitial water of sediment from its habitat. Rates of removal are approximately half the rates observed for neutral amino acids (Table 1) when expressed in comparable units. Two explanations for this lower rate can be suggested. First, glu is present in interstitial water and therefore contributes to total primary amine but is relatively unavailable to the animal. Secondly, 15–30% of the primary amines in interstitial water are not retained on Dowex-50 in the acid phase and may represent material, some or all of which is unavailable for transepidermal uptake.

Comparison of the amino acids present in interstitial water before and after exposure to *Dendraster* shows a change in total primary amines and a change in pattern of amino acids present (Fig. 4). The changes are consistent with predictions based on experimental results with single amino acids. Thus, neutral amino acids are reduced, while glu is relatively unchanged; total primary amines are reduced to a stable level of 5–7 μM.

As noted, a portion of the primary amines normally present in interstitial water does not appear to behave as typical amino acid. Changes in the contribution of this fraction to total primary amines during exposure to *Dendraster* were not determined. Estimation of its concentration by difference before and after passing through a Dowex-50 column proved to be unsatisfactory. Presence of this unknown material also prohibits a complete description of the primary amines which appear in the medium in which animals are incubated.

Amino acids removed from solution by these animals apparently enter metabolic pathways. In general, acidification of the medium leads to a reduction in measured radioactivity of a medium sample after an animal has been exposed to a known labelled substance. This acid volatile radioactivity may be evidence for the production of $^{14}CO_2$, commonly found in experiments of this kind (Stephens, 1972).

The presence of radioactivity which is not acid volatile and which is not amino acid at the end of incubation experiments may be evidence of the presence of labelled metabolites lost from the animals. These have not been identified but are not primary amines.

The failure of *Dendraster* to remove gylcylglycine from dilute solution suggests that if epidermal digestion does occur in these animals, it is a slow process compared to transepidermal transport of amino acids. This is consistent with the very low protein digestion activities reported by Péquignat (1970), but other pathways of disappearance of glycylglycine cannot be excluded in these observations.

Possible bacterial contributions to the appearance of labeled, acid volatile and acid nonvolatile metabolites in the medium and to the disappearance of glycylglycine cannot be completely excluded. However, bacterial contributions to influx and net flux measurements are certainly small. Animals were incubated for 24 hours in penicillin (500,000 units/liter) and streptomycin (200 mg/liter) and rates of influx and net flux of lysine and serine compared to unincubated controls. No difference was observed. Such incubation would not inactivate all possible microbial contaminants, but one would anticipate some effect on rates if microbial activity plays a substantial role in these observations. Failure to observe influx of glutamate in *Dendraster* also suggests that the animal is the principal agent; there is no reason to expect that glutamate would not be metabolized as well as other amino acid substrates by a contaminant microbial population.

The potential contribution of transepidermal transport to the animals can be estimated by comparing rates of influx to an estimate of reduced carbon required to support oxidative metabolism. An approximate conversion factor to equate oxygen consumption with complete oxidation of a mixture of amino acids (1 ml O_2 = 1 mg amino acid) and an average molecular weight for amino acids of 100 can be used. Then, the average oxygen consumption of 4.3 μl $O_2/(hr \cdot cm^2)$ is equivalent to 43 nmoles amino acid/$(hr \cdot cm^2)$. The average influx of naturally occurring amines from interstitial water (Table I) is 24.9 nmoles/$(hr \cdot cm^2)$, a contribution of 58% of the material required to support oxygen consumption. This estimate is probably based on an overly conservative figure for the level of naturally occurring amines in the sediment. Only two of the fifteen cores analyzed from the habitat showed less than 50 μmoles primary amines (17,36 μmoles) in the 0–3 cm zone of the sediment. The average was 115 μM. Since the K_t for influx of gly was measured as 74 μM (Fig. 3), the presence of levels of primary amines in interstital water greater than the 20 μM used for this estimate would certainly lead to greater influx rates and an increased contribution to carbon requirements. In fact, it can be concluded that if the surface of *Dendraster* is exposed to levels of primary amines measured in 14 or our 15 samples ($\geq$ 35 μM), influx is sufficient to account for oxygen consumption.

This discussion assumes that the bulk concentration of primary amines measured in the interstitial water of the sediment is a measure of concentrations available at the surface of the animal. Stephens (1975) has reported increased primary amines in interstitial water as a result of irrigation by the annelid infauna. This may also be true for *Dendraster*. Alternatively, renewal of primary amines at the surface may be dependent on bulk flow of interstitial water and diffusion. Until this ques-

tion can be investigated, it should be re-emphasized that the conclusions of the preceding paragraph should be phrased conditionally.

Timko (1976) describes suspension feeding in *Dendraster*. When behaving in this fashion, about one-third of the anterior portion of the test is embedded in the sediment substrate. Clearly, only a portion of the test would be in contact with interstitial water of the sediment in this feeding mode. *Dendraster* also behaves as a prone deposit feeder according to Timko and other authors. In this feeding mode, the animal is often below the sediment surface and is fully exposed to interstitial water. Animals in both the inclined suspension feeding and prone deposit feeding orientation were observed in the shallow water population at Point Mugu Naval Base.

Timko (1976) concludes that *Dendraster excentricus* is primarily a suspension feeder. However, Chia (1969) reports that all the individuals in a population from Puget Sound, Washington, were completely buried at low tide. We suggest that *Dendraster* can supplement both suspension feeding and deposit feeding by influx of amino acids into the epidermis. This supplement would be small when the animals are behaving as inclined suspension feeders but would be large during deposit feeding. In our experiments, transepidermal influx of amino acids would support energy metabolism at ambient levels of primary amines greater than 35 μM; our measurements indicate these are realistic levels for prone deposit feeding animals buried in the superficial layers of the sediment.

Our data suggest that animals might indeed survive without taking in and digesting food, provided *Dendraster* has pathways for translocating nutrients from the epidermis to deeper tissues. However, it is more likely that transepidermal uptake of small organic compounds may contribute to the sustenance of the epidermis. If there is a barrier to translocation of nutrients in *Dendraster* comparable to that reported for other asteroids and echinoids, direct uptake of nutrients from the environment may play a large role in the nutrition of pedicellariae, spicules, podia and other epidermal structures. Our data suggest that the oxidative requirements of the epidermis represent a large fraction of the total requirements of the animal. However, levels of ambient primary amines (> 35 μM) adequate to support total oxidative metabolism are *a fortiori* adequate for the epidermal fraction thereof.

Summary

1. Influx of amino acids from dilute solution into the sand dollar, *Dendraster*, was measured by following the disappearance of radioactivity in the medium supplying known labeled substrates. Net flux was monitored simultaneously by following the decrease in primary amines in the medium fluorometrically. Rates of influx and net flux correspond closely at ambient concentrations greater than 5 μM.

2. *Dendraster* is capable of net accumulation of some of the primary amines normally found in the interstitial water of its sediment habitat.

3. A sensitive method for location of amino acids on thin layer chromatograms is described. Comparison of interstitial water before and after exposure to *Dendraster* shows a changed pattern of amino acids, as well as a decrease in total amino acids, which is consistent with measurements of rates of influx with single substrates.

4. Comparison of rates of influx of naturally occurring primary amines with the metabolic requirements of animals as estimated from their oxygen consumption indicates that *Dendraster* can acquire sufficient reduced carbon to account for its oxidative needs if its surface is exposed to naturally occurring primary amines at concentrations greater than or equal to 35 μM.

5. Primary amines in the interstitial water of sediment in the immediate vicinity of a shallow water population of *Dendraster* range in concentration from 17 to 244 μM ($115 \pm 60\ \mu$M).

6. *Dendraster* lives in an environment which is relatively rich in amino acids, and it possesses a transport system which can accumulate these compounds at rates sufficient to provide a significant supplement to other forms of feeding. These findings support the hypothesis that sustenance of epidermal structures of echinoids and asteroids may be relatively independent of translocation of nutrients from the digestive organs and may be based primarily on transepidermal influx of nutrients from the medium.

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